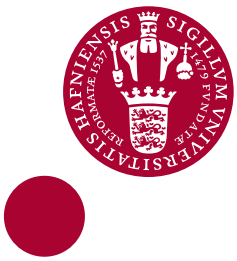

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Batch medication of intestinal infections in nursery pigs—A randomised clinical trial on the efficacy of treatment strategy, type of antibiotic and bacterial load on average daily weight gain

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ABSTRACT

Introduction: Previous research projects have demonstrated the need for better diagnostic tools to support decisions on medication strategies for infections caused by *Escherichia coli* F4 (F4) and F18 (F18), *Lawsonia intracellularis* (LI) and *Brachyspira pilosicoli* (PILO). This study was carried out as a randomised clinical trial in three Danish pig herds and included 1047 nursery pigs, distributed over 10 batches and 78 pens. The objectives of this study were: (1) to assess the effect of four 5-day treatment strategies (initiated at clinical outbreak of diarrhoea or at fixed time points 14, 21, or 28 days after weaning) on average daily weight gain (ADG); (2) to compare the effect of treatment with doxycycline or tylosine on diarrhoea prevalence, pathogenic bacterial load, and ADG; (3) to evaluate PCR testing of faecal pen floor samples as a diagnostic tool for determining the optimal time of treatment.

Results: (1) The four treatment strategies had a significant overall effect on ADG ($p = 0.01$). Pigs starting treatment 14 days after weaning had a significantly higher ADG (42 g) compared to pigs treated on day 28 ($p = 0.01$).

(2) When measured 2 days after treatment, doxycycline treatment resulted in fewer LI-positive pens ($p = 0.004$), lower excretion levels of LI ($p = 0.013$), and fewer pens with a high level of LI ($p = 0.031$) compared to pens treated with tylosine. There was no significant difference in F4, F18 and PILO levels after treatment with the two antibiotic compounds. There was a significant difference ($p = 0.04$) of mean diarrhoea prevalence on day 21 of the study between pens treated with tylosine (0.254, 95% CI: 0.184–0.324), and doxycycline (0.167, 95% CI: 0.124–0.210). The type of antibiotic compound was not found to have a significant effect on ADG ($p = 0.209$).

(3) Pigs starting treatment on day 14 in pens where F4, F18, LI or PILO were detected by qPCR on the pen floor had a statistically significant increase in ADG (66 g) compared to pigs treated on day 14 in pens where no enteric pathogens were detected ($p = 0.04$).

Conclusions: The results of this study showed that the highest ADG was achieved when treatment was initiated 14 days after weaning in pens where intestinal pathogens were detected. Doxycycline was more effective in reducing diarrhoea and LI excretion levels than treatment with tylosine.

1. Introduction

The use of antimicrobials in livestock production is subject to continued debate due to the risk of bacterial resistance spreading to the human population (van den Bogaard and Stobberingh, 2000; Leung et al., 2011; Marshall and Levy, 2011). Denmark has a large pig industry, and antimicrobials prescribed for pigs account for 78% of the total usage for animals, corresponding to 84.8 t of

Abbreviations: qPCR, quantitative polymerase chain reaction; ADG, average daily weight gain (from 14 to 35 days after weaning); F4, *Escherichia coli* F4; F18, *Escherichia coli* F18; LI, *Lawsonia intracellularis*; PILO, *Brachyspira pilosicoli*; S1, treatment strategy 1; S2, treatment strategy 2; S3, treatment strategy 3; S4, treatment strategy 4.

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active compound (DANMAP, 2014). In 2012, 43% of active compounds prescribed for pigs were used in nursery pigs (7–30 kg live weight), of which 75% were prescribed for oral treatment of gastrointestinal disease (Jensen et al., 2014). There is great potential to reduce the total antibiotic usage by improving diagnostic criteria for batch medication of intestinal infections in nursery pigs, thereby avoiding unnecessary treatments. The most common method of treating intestinal disease in Danish nursery pigs is oral treatment for 5 days with either doxycycline or tylosine (Hybschmann et al., 2011; DANMAP, 2014; Jensen et al., 2014; EMA, 2015).

Diarrhoea has been shown to have a weak association to intestinal infection and therefore also for decisions to initiate antibiotic batch medication in pigs. Previous studies have demonstrated that bacterial enteric infections can be present within a group of pigs before the disease is clinically evident to farmers and veterinarians (Brandt et al., 2010; Paradis et al., 2012; Weber et al., 2015). It has also been demonstrated that some groups of nursery pigs experience clinical diarrhoea of non-bacterial aetiology and therefore should not be treated with antibiotics (Callesen et al., 2007; Chase-Topping et al., 2007; Thomson, 2009; Pedersen et al., 2014a). The mean bacterial load determined by qPCR testing of a pooled faecal sample for *Escherichia coli* F4 (F4) and F18 (F18), *Lawsonia intracellularis* (LI) and *Brachyspira pilosicoli* (PILO) from a group of nursery pigs can be used to determine the prevalence of bacterial enteritis/colitis (Pedersen et al., 2014b). These findings have made it possible to classify outbreaks as high or low pathogenic diarrhoea (Pedersen et al., 2014a). By classifying the pathogenicity of diarrhoeic outbreaks, it is possible to explore whether PCR testing of pooled faecal samples can be used as a decision tool for initiating batch medication.

The aim of this study was to improve the effect of antibiotic treatment for enteric infections in groups of nursery pigs. We determined the optimum time-point for initiation of batch medication, as well as the diagnostic value of using PCR testing of pooled faecal samples at the potential time of treatment. Furthermore, we compared the efficacy of batch medication with doxycycline and tylosine. The efficacy of treatment was measured as reduced diarrhoea prevalence, pathogenic bacterial load and average daily weight gain (ADG). Three different objectives were investigated in the study: **Objective 1** was to assess the effect of four 5-day treatment strategies on ADG and pathogenic bacterial load, initiated either at clinical outbreak of diarrhoea or at fixed time points 14, 21, or 28 days after weaning; **Objective 2** was to compare the effect of doxycycline and tylosine treatments on diarrhoea prevalence, pathogenic bacterial load, and ADG; **Objective 3** was to evaluate PCR testing of faecal pen floor samples at the time of treatment as a diagnostic tool for determining the optimal treatment time.

2. Methods

The study was performed as a clinical field trial approved by the Danish Medicines Agency (License no. 2013110114). Data were collected from January 2014 until October 2014.

2.1. Design

The study was a non-blinded randomised, controlled clinical trial in three herds, with a 2×4 factorial design with two antibiotics and four treatment strategies. The groups were allocated by cluster randomisation. The unit of randomisation was the pen, and the experimental unit was either the individual pig or the pen, depending on the outcome. A batch was defined as a group of nursery pigs all weaned at the same time into the same section. A total of two to four batches per herd were included 14 days after weaning and followed for 21 days. Batches with mixed age groups or treatments of unexpected diseases were excluded.

Table 1
Distribution of pens per herd and per treatment strategy.

		Herd			
Strategy	Antibiotic	1	2	3	Pens per treatment strategy
S1	Doxycycline	4	4	2	10
S1	Tylosine	4	4	2	10
S2	Doxycycline	4	4	2	10
S2	Tylosine	4	4	2	10
S3	Doxycycline	5	4	2	11
S3	Tylosine	5	4	2	11
S4	Doxycycline	4	3	1	8
S4	Tylosine	4	3	1	8
Pens per herd		34	30	14	

2.2. Sampling procedures

A total of 78 pens were included in the study (Table 1). Within a batch, four double pens sharing the same feeder were randomly selected. A total of 15 pigs from each of the selected double pens were selected by systematic random sampling. If there were fewer than 15 pigs in the selected pen, all pigs were selected. All trial pigs were ear-tagged with a unique ID number. Pooled faecal pen floor samples were collected from each study pen at day 14, 21, 28 and 35 post weaning. Excretion level of F4, F18, LI and PILO analysed by qPCR in the pooled faecal samples was used to evaluate pathogenic bacterial load.

To address Objective 1, randomly selected double pens were allocated to four different treatment strategies. The four strategies tested were: **strategy 1 (S1)**: 5 days of antibiotic treatment initiated 14 days after weaning; **strategy 2 (S2)**: 5 days of antibiotic treatment initiated 21 days after weaning, or at an earlier time point if there was an outbreak of clinical diarrhoea; **strategy 3 (S3)**: 5 days of antibiotic treatment initiated 28 days after weaning, or at an earlier time point if there was an outbreak of clinical diarrhoea; **strategy 4 (S4)**: 5 days of antibiotic treatment only initiated in response to an outbreak of clinical diarrhoea. An outbreak of clinical diarrhoea was defined by the fulfilment of one of the following criteria: >=50% of pigs with diarrhoea; >50% of pigs treated individually for intestinal disease. Regardless of predetermined time point for treatment all pens were treated for animal welfare reasons when a diarrheic outbreak occurred.

To address Objective 2, two different active compounds (doxycycline/tylosine) were used in parallel throughout the study. Pens were assigned at random to antibiotic type when included at the start of the study. To address Objective 3, the qPCR test results from samples collected at the day of treatment 14 days after weaning (S1) were used to classify the study pens according to the load of pathogenic bacteria in the pooled faecal pen floor sample collected on the day that treatment was initiated. This classification was used in the subsequent statistical analysis to assess the effect of faecal bacterial intestinal pathogens at the day of initiation of treatment on ADG in the following 21 days.

2.3. Sample size considerations

Sample size calculations were performed using formulae for differences in mean between two groups. The groups were allocated by cluster randomisation (at pen level), but weight gain was measured in the individual pig. The study was designed to detect a 50 g ADG difference between pigs subjected to different treatment strategies. When taking into account the effect of clustering (as described by Dohoo et al., 2009), each treatment strategy required 180 pigs (Dohoo et al., 2009).

2.4. Herds

Potential study herds were selected from herds serviced by two veterinary practices in the eastern part of Denmark. Herds free of porcine reproductive and respiratory syndrome virus, Edema disease, *Brachyspira hyodysenteriae*, salmonellosis, atrophic rhinitis, and other acute diseases with a risk of medication were included. Vaccination against LI was an exclusion criterion. High pathogenic diarrhoea was an inclusion criterion and was defined as an outbreak with ≥1.5 diarrhoeic pools per pen and faecal pool samples containing ≥35.000 bacteria per g of faeces, calculated as the sum of F4, F18, PILO and LI per g faeces (Pedersen et al., 2014a). Three herds were included in the study. All herds had all-in all-out batch production in sectioned compartments with 2300 to 3600 pen places per herd. The flooring consisted of one third solid floor and two thirds slatted floor. Pigs per pen ranged for 10–40 pigs. Pig density and layout of pens was similar in all three herds. The feed fulfilled the Danish nutrient standards (SEGES-VSP, 2015) and was home-mixed, formulated with wheat, barley and soybean-meal as the main ingredients. The nursery pigs were crossbred Yorkshire/Landrace and Duroc. All three herds were participating in the Danish Specific Pathogen Free system (SPF); (SPF-sus, 2015) and were all declared free of *Actinobacillus pleuropneumoniae* type 2, 6 and 12, porcine reproductive and respiratory syndrome virus, mange mites and lice. Herds 1 and 2 were declared free of *Mycoplasma hyopneumoniae*, unlike Herd 3, which was positive and using a vaccination programme to control the infection. To control post-weaning colibacillosis, all herds used 3000 ppm zinc oxide in the feed for the first 14 days after weaning. During the field trial, each herd was visited for clinical examination once a week. All pigs were weighed at the start of the trial (14 days after weaning) and at the end of the trial (35 days after weaning), using a scale ("Bjerringbrovægt 1298GE") with a precision of 100 g.

2.5. Assessment of faecal consistency

Faecal samples were collected from each pig by digital rectal manipulation using a gloved hand at the start of the study, on the day the pigs were treated and at the end of the study. The faecal samples were scored by one observer using a faecal consistency scale with four categories, where scores 1 and 2 represented normal faeces and score 3 and 4 represented diarrhoea (Pedersen and Toft, 2011).

2.6. Laboratory analyses

Pooled faecal pen floor samples were collected by swiping a gloved hand over the slatted floor once a week from every study pen, and the collected faeces were stored in sealed plastic containers. Cooled samples were transported (in a polystyrene box with freezing elements) to the Danish National Veterinary Institute for further laboratory analyses. Approximately 1 g of the pooled faecal samples was homogenised in a stomacher for 1 min with phosphate buffered saline (PBS) to obtain a 10% (w/v) faecal suspension. An aliquot of the suspension was transferred to a 2 ml microfuge tube and stored in a freezer at minus 20 °C until DNA extraction, as previously described by (Pedersen et al., 2012). DNA was stored in a minus 20 °C freezer until the F4, F18, PILO and LI content was quantified by qPCR, as previously described by (Stahl et al., 2011) with the exception that standard curves for quantification were prepared from DNA extracted from faeces spiked with 10-fold dilution series of the corresponding pathogen, using the same extraction procedure as for the faecal specimens (Pedersen et al., 2012). Detection limits per gram faeces were 5.7×10^4 colony-forming units (CFU) for F4, 1.5×10^3 CFU for F18, 2×10^3 bacteria for LI and PILO. Linear ranges were 5.7×10^9–5.7×10^5 CFU/g faeces for F4, 1.5×10^{10}–1.5×10^5 CFU/g faeces for F18, 2×10^8–2×10^4 bacteria/g faeces for LI and 2×10^8–2×10^4 bacteria/g faeces for PILO.

2.7. Treatments

Doxycycline hydrate was used in the trial at a dosage of 12.5 mg per kg bodyweight, as recommended by the supplier (Soludox Vet.®, Dechra Veterinary Products A/S). Tylosine tartrate was used in the trial at the recommended dosage of 7.5 mg per kg bodyweight (Tylan® Vet., ELANCO Animal Health). Both antibiotics were administered via a water trough at pen level. The daily dose was divided equally over two daily administrations in order to increase the chances of uniform dosing. Due to legal regulation, pigs had access to fresh water via drink nipples during the treatment period. Oxytetracycline (Engemycin® Vet. 100 mg/ml, MSD Animal Health), at a standard dose of 10 mg per kg bodyweight was used for 3 days for individual treatment of intestinal disease in pens allocated to pen treatment by doxycycline. Oxytetracycline was chosen because no doxycycline products were registered for injection in Denmark. In study pens allocated to pen treatment by tylosine, any individual cases of intestinal disease were treated using tylosine (Tylan® Vet. 200 mg/ml, ELANCO Animal Health) with a standard dose of 10 mg per kg bodyweight for 3 days. Throughout the study period, all pen treatments were performed according to the study protocol. Pen treatments were initiated after the predetermined day according to protocol, or at an outbreak of clinical diarrhoea defined by the fulfilment of one of the following criteria: >=50% of pigs with diarrhoea or >50% of pigs individually treated for intestinal disease. The farmer/stockman was allowed to treat individual pigs with clear clinical signs of intestinal or other diseases. The criteria for individual treatment of intestinal disease were: observed defecation of watery faeces; a line of watery faeces in the anal region; marked loss of body condition. If the disease progressed, the pigs were weighed and removed from the study.

2.8. Statistical analyses

Statistical analyses were performed in R version 3.1.2 (R-Core-Team, 2014), with mixed models implemented using the lme4 and lmerTest packages (Bates et al., 2015; Kuznetsova et al., 2015). The effect of the four treatment strategies for 5-day treatments with doxycycline or tylosine (Objectives 1 and 2) was assessed using a mixed linear model (model 1) with ADG as the outcome, and pen, batch and herd as random effects to account for clustering at pen, batch and herd level. ADG was calculated by subtracting the start weight (bodyweight on day 14) from the end weight (bodyweight on day 35). Least squares means (lsmeans) included in the lmerTest package were used to summarise the effect of the explanatory variables on the outcome in the mixed model. Start weight, gender, faecal status, bacterial load, treatment strategy (S1–S4) and antibiotic group (doxycycline/tylosine) were individually screened as potential explanatory variables by univariable linear regression. Candidate variables with a p-value < 0.1 were used in the multivariable linear model to investigate the association with ADG. Previous eliminated variables were reintroduced to the model to control for confounding. A variable was considered to be a confounder if the estimates of the significant variables changed by 25%. Eliminated variables that were identified as confounders were retained in the final model. The measurement of difference in pathogenic load of pathogenic bacteria between strategy group and antibiotic group was tested by Kruskal-Wallis rank sum test.

The association between demonstration of faecal bacterial intestinal pathogens and ADG (Objective 3) was analysed using data from pens selected for S1 to build a mixed linear model (model

Table 2
Detection and excretion levels of pathogens in faecal pen floor samples by qPCR, and diarrhoea prevalence in non-treated pens.

Days after weaning	14		21		28		35	
Pathogen detected								
E. coli F4	2/78[a]	2.6%[b]	1/58	1.7%	0/31	0%	0/13	0%
E. coli F18	40/78	51.3%	26/58	48.3%	4/31	12.9%	0/13	0%
L. intracellularis	15/78	19.2%	23/58	39.7%	23/31	74.2%	12/13	92.3%
B. pilosicoli	4/78	5.1%	6/58	10.3%	5/31	16.1%	2/13	15.4%
No pathogen detected	32/78	41.0%	11/58	19.0%	5/31	16.1%	1/13	7.7%

Excretion level of positive samples (log10 bacteria/g faeces)	Quartiles			Quartiles			Quartiles			Quartiles		
	25%	50%	75%	25%	50%	75%	25%	50%	75%	25%	50%	75%
E. coli F4	5.55	5.66	5.82	–	5.98	–	–	–	–	–	–	–
E. coli F18	5.62	5.96	6.38	5.40	6.41	7.27	6.69	7.13	8.00	–	–	–
L. intracellularis	3.16	4.01	5.07	3.61	4.76	5.51	4.79	5.85	7.01	6.09	6.70	7.03
B. pilosicoli	2.98	3.38	3.96	3.19	3.61	3.89	3.09	4.46	5.12	4.33	4.72	5.12
Pen-level diarrhoea%	0.00	0.09	0.18	0.13	0.27	0.40	0.20	0.27	0.35	0.25	0.45	0.50

Results of qPCR analysis and diarrhoea prevalence from pens examined weekly at day 14, 21, 28, and 35 post weaning. All pen floor samples collected before an antibiotic treatment was labelled as samples from non-treated pens. Pen floor samples collected after treatment are not included.
[a] Pens with pathogen detection.
[b] Prevalence of positive pens.

2) with ADG as the outcome, and pen, batch and herd as random effects. The qPCR results from the pen samples and antibiotic group (doxycycline/tylosine) were the primary independent variables and were included as fixed effects. Assumptions for linear regression were assessed visually using normal quartile plots of residuals.

3. Results

3.1. Descriptive results

Two batches from herd 3 were excluded due to a fire in the herd facilities, and four pens were excluded due to a failure to record diarrhoeic outbreaks. Data from 12 pigs were missing due to death or movement to other sections. A total of ten batches (four batches from herd 1 and 2 and two batches from herd 3) with 78 pens containing 1047 pigs from the three study farms were included in the statistical analyses. The average number of pigs per pen was 23.7 (min = 10, max = 40). Twenty pens were selected for S1 and all were treated 14 days after weaning. Twenty pens were selected for S2, and 18 of these pens were treated 21 days after weaning, while treatment in two pens was initiated an earlier time point due to a clinical outbreak of diarrhoea. A total of 22 pens were selected for S3 and 17 of these were treated 28 days after weaning, while treatment was initiated at an earlier time point due to a clinical outbreak of diarrhoea in five pens. Sixteen pens were selected for S4. No treatment was initiated in 13 of these, while in the remaining three pens, treatment was initiated due to a clinical outbreak of diarrhoea.

3.1.1. Excretion of intestinal pathogenic bacteria and diarrhoea prevalence

Diarrhoea prevalence, detection and excretion level of F4, F18, LI and PILO in pen floor faeces sampled once a week from non-treated pens are shown in Table 2. In all three herds, F18 and LI were the most frequently detected pathogens. Initially, F18 was the predominant pathogen, but LI was more common at the end of the study period. F18 was most frequently detected on day 14 (51.3%) and day 21 (48.3%), and LI were detected on day 14, 21, 28 in 19.2%, 39.7%, 74.2% and 92.3% pens, respectively. PILO was detected at low frequency over time, and F4 was rarely found. No pathogen was detected on day 14, 21, 28 and 35 in 41.0%, 19.0%, 16.1% and 7.7% of pens, respectively. The diarrhoea prevalence at pen level increased over time from a median diarrhoea prevalence of 0.09 on day 14–0.45 on day 35. The excretion level from positive samples showed an increase over time for all four pathogens.

Table 3 shows the total bacterial load of the most frequently detected pathogens (F18 and LI) stratified by strategy and antibiotic groups. The total bacterial load was calculated at pen level by the sum of four faecal pen floor samples collected weekly on day 14, 21, 28, and 35 post weaning. The total excretion level of F18 was significantly higher in S4 compared to the other strategy groups. There was no significant difference of median bacterial load of F18 between the two antibiotic groups ($p = 0.703$). There was no significant difference in the median total bacterial load of LI between the four strategy groups ($p = 0.335$), whereas there was a significant difference ($p = 0.03$) in the median total bacterial load of LI between the two antibiotic groups (doxycycline = $10^{4.79}$ LI bacteria/g faeces, tylosine = $10^{6.08}$ LI bacteria/g faeces).

3.2. Analytical results

3.2.1. Effect of treatment strategy and type of antibiotic on average daily weight gain

The estimates from the final model for ADG (Model 1) and least squares means of ADG in strategy and antibiotic groups are presented in Table 4. The variables included start weight, strategy and antibiotic group. Antibiotic group was included despite failing to meet the univariate criteria for inclusion ($p < 0.1$) because it was our primary variable of interest. Average daily weight gain was significantly correlated with strategy group ($p = 0.01$), with the highest ADG observed in S1 and the lowest in S4. There was a significant difference in ADG between S1 and S3 and S4, as tested by least squares means. The ADG of pigs selected for S3 and S4 were 42 g and 56 g lower, respectively, than pigs selected for S1. Pigs treated with tylosine had an apparent decrease in ADG of 15 g compared to pigs treated with doxycycline, but this difference was not statistically significant ($p = 0.209$). The intraclass correlation coefficient (ICC) values showed a 12.3% variation between the three herds, which meant that most of the variation (87.7%) was within herds. To control for the effect of pens treated due to clinical outbreak at an earlier time point then predetermined by the strategy groups on ADG, the model was run again where these pens were excluded. The estimates of the reduced model did not change markedly that could interfere on the conclusions of the effect of the main variables, strategy and antibiotic group on ADG.

Table 3
Total bacterial load of *L. intracellularis* and *E. coli* F18.

Total bacterial load (bacteria/g faeces)*	*L. intracellularis*				*E. coli* F18			
		Quartiles				Quartiles		
Treatment strategy group	Positive/total pens	25%	50%	75%	Positive/total pens	25%	50%	75%
S1	16/20	4.65	5.68[a,**]	7.48	18/20	5.71	6.26[a]	6.74
S2	13/20	5.17	6.08[a]	7.71	18/20	5.49	5.92[a]	6.72
S3	20/22	4.48	6.02[a]	7.52	15/22	5.61	5.97[a]	6.87
S4	14/16	5.82	6.95[a]	7.55	12/16	6.77	6.98[b]	7.55
Antibiotic group								
Doxycycline	22/33	3.73	4.79[a]	6.16	28/33	5.67	6.39[a]	6.88
Tylosine	29/32	4.99	6.08[b]	6.83	26/32	5.61	5.92[a]	6.75

* = Total bacterial load calculated by the sum of four faecal pen floor samples collected weekly at day 14, 21, 28, and 35 post-weaning from positive pens (log10 bacteria/g faeces).
** = Different letter indicates significant difference ($p < 0.05$) tested by Kruskal-Wallis rank sum test.

Table 4
Estimates for fixed effects, intraclass correlation coefficients (ICC) for random effects and estimated means from a linear mixed model for average daily weight gain from 14 to 35 days after weaning (kg).

Fixed effects	Estimate (β_x)	Std. error	95% CI	p-value	Least squares means	SEM
Intercept	0.131	0.042	0.026; 0.237	0.029		
Start weight	0.045	0.002	0.026; 0.237	<0.000		
Strategy group				0.010		
S1	–				0.552[a,*]	0.037
S2	−0.028	0.016	−0.060; 0.011	0.086	0.524[ab]	0.037
S3	−0.042	0.016	−0.074; −0.011	0.0106	0.510[b]	0.038
S4	−0.056	0.017	−0.091; −0.022	0.002	0.496[b]	0.038
Antibiotic group				0.209		
Doxycycline	–				0.528[a]	0.036
Tylosine	−0.015	0.012	−0.038;0.008		0.513[a]	0.036
Random effects	Variance	Std. dev.	ICC(%)			
Herd	0.002	0.044	12.3			
Batch	0.001	0.026	4.3			
Pen	0.004	0.059	22.6			
Residuals	0.009	0.100	60.5			

Model 1. "–" Indicates reference.
* = Different letter indicates significant difference ($p < 0.05$) tested by lsmeans.

3.2.2. *Faecal excretion of* Lawsonia intracellularis *and diarrhoea prevalence after treatment with doxycycline and tylosine*

Faecal excretion of LI analysed by qPCR from pooled pen floor samples is shown in Table 5. On the day of treatment initiation, LI was detected in 12 of the 33 pens treated with doxycycline (median excretion = $10^{5.8}$ LI bacteria/g faeces) and in 14 of the 32 pens treated with tylosine (median excretion = $10^{5.7}$ LI bacteria/g faeces). At the second sampling (i.e. 2 days after treatment), LI was detected in 7 of the 33 pens treated with doxycycline (median excretion = $10^{3.5}$ LI bacteria/g faeces) and in 18 of the 32 pens treated with tylosine (mean excretion = $10^{5.8}$ LI bacteria/g faeces). There was a significant association ($p = 0.003$) of detection of LI (>2×10^3 bacteria/g faeces) 2 days after treatment between the two antibiotic groups with an odds ratio of 4.78 (95% CI: 1.67–14.96) in pens treated with tylosine. There was also an association ($p = 0.03$) of detection of high LI levels (>10^6 bacteria/g faeces) 2 days after treatment between the two treatment regimens with an odds ratio of 10.67 (95% CI: 1.78–204.83) in pens treated with tylosine. There was also a significant difference ($p = 0.04$) between pens treated with tylosine (0.254, 95% CI: 0.184–0.324), and doxycycline (0.167 95% CI: 0.124–0.210) in the mean prevalence of diarrhoea on the final day of the study.

3.2.3. *Association between bacterial intestinal pathogen load at initiation of treatment and ADG*

Table 6 shows the estimates for the mixed linear model with mean ADG as outcome (Model 2). Data for this model are a subset of the whole dataset of pigs treated on day 14 after weaning (S1). In the final mixed linear model, the qPCR results for detection of bacterial intestinal pathogens were dichotomised into positive or negative results to ensure a sufficient number of pigs were included in each group for the analysis. A qPCR sample was classified as positive if the sample was positive for one or more of the four analysed pathogens. After adjusting for herd, batch, pen, start weight and type of antibiotic treatment, there was a significant difference in ADG between qPCR negative and positive pens ($p = 0.040$). Pigs treated in pens with a positive qPCR pen sample had an ADG increase of 66 g compared to pigs treated in pens with a negative qPCR sample.

4. Discussion

The main finding of this study was that the time of treatment affected the ADG. In general, the earlier pigs were treated (starting 14 days after weaning), the higher the ADG; pigs treated 14 days after weaning (S1) had significantly higher ADG than pigs treated on day 21 or day 28. All pigs in S1 were treated 14 days after weaning, and no other clinical parameters were taken into account when deciding upon the initiation of the pen treatment. The pigs treated on day 14 were characterised by having the lowest diarrhoea prevalence at pen level and a lower level of intestinal pathogenic bacteria at the day of treatment initiation compared to pigs treated on day 21 or 28. This study also demonstrated the effect of detecting intestinal pathogens on the day of treatment initiation on the ADG of the pigs treated. Pigs housed in pens where no pathogens were detected on the day of treatment initiation had a significantly lower ADG than those housed in pens where one or more pathogens were detected. Overall, these findings show that antimicrobial treatment had the greatest effect on ADG in the pens where pigs excreting intesti-

Table 5
Faecal excretion of *Lawsonia intracellularis* analysed by qPCR from pooled pen floor samples.

	Doxycycline	Tylosine	p-value	Odds ratio
L. intracellularis detection at initiating treatment				
Positive pens	12/33	14/32	0.723[a]	
Median excretion of positive pens (bacteria/g faeces)	$10^{5.8}$	$10^{5.7}$	0.837[b]	
L. intracellularis detection 2 days after last treatment (<2×10^3 bacteria/g faeces)				
Positive pens	7/33	18/32	0.004[a]	4.78
Median excretion of positive pens (bacteria/g faeces)	$10^{3.5}$	$10^{5.8}$	0.013[b]	
Detection of high level of *L. intracellularis* (>10^6 bacteria/g faeces) excretion 2 days after treatment				
Positive pens	1/33	8/32	0.031[a]	10.67
Reduction of *L. intracellularis* excretion from initiation of treatment and 2 days after treatment[c]				
Pen with reduction	11/12	7/14	0.06[a]	0.09

Pens were randomly selected for treatment with 5 days of doxycycline or 5 days of tylosine.
a = Chi^2-test, b = Kruskal-Wallis rank sum test, c = faecal excretion of *Lawsonia intracellularis* was measured on the day of the first treatment and again 2 days after the last treatment.

Table 6
Estimates for fixed effects, intraclass correlation coefficients (ICC) for random effects and estimated means from a linear mixed model for average daily weight gain from 14 to 35 days after weaning (kg) in pigs selected for Treatment strategy 1 (S1).

Fixed effects	Estimate (βx)	Std. error	95% CI	p-value	Least squares means	SEM
Intercept	0.009	0.056	−0.103; 0.136	0.873		
Start weight	0.053	0.004	0.044; 0.061	<0.000		
qPCR pen sample				0.040		
Negative	–				0.503	0.036
Positive	0.066	0.030	0.002; 0.136		0.569	0.031
Treatment group				0.557		
Doxycycline	–				0.543	0.032
Tylosine	−0.013	0.021	−0.058; 0.033		0.520	0.032
Random effects	Variance	Std. dev.	ICC(%)			
Herd	0.002	0.043	12.7			
Batch	0.001	0.038	11.5			
Pen	0.002	0.040	15.0			
Residual	0.007	0.086	60.8			

Model 2: Data from a subset of the whole dataset of pigs selected for Treatment strategy 1 (S1).
"–" Indicates reference.

nal pathogen were treated early, when clinical diarrhoea was still at a low level. This is in accordance with previous findings that have shown subclinical enteric infections to be common, so using clinical diarrhoea in the decision to initiate treatment can be problematic (Jacobson et al., 2003; Paradis et al., 2012; Weber et al., 2015). Interestingly, when treatment was initiated in pens without pathogenic bacteria, the pigs performed poorer than pigs in the pens where pathogenic bacteria were detected. The most plausible explanation for this finding might be that the antibiotics were used at a time point where they did not affect intestinal pathogens. Only 5 days of antibiotic treatment was used throughout the study period. Therefore, pens treated at day 14 and without detection of pathogenic bacteria could have experienced intestinal infections after the antibiotic treatment that might have resulted in a lower ADG. The ADG of pigs treated at day 14 without any detectable pathogens were at a similar level as pigs treated at day 28 or later which may support the idea that these pens were treated before an infection occurred in the pigs. This demonstrates the diagnostic value of testing faecal pen floor samples at the time of treatment as a tool for initiating antibiotic treatment. Due to the small sample size, it was not possible to investigate the effect of different excretion levels of pathogenic bacteria on ADG, thereby determining a critical threshold of pathogenic bacteria excretion. If qPCR analyses can be performed in real-time at herd facilities in the future, it may be possible to determine a critical threshold for the level of pathogenic bacteria excretion for the initiation of treatment.

In this study, there was no significant difference in ADG between pigs treated with doxycycline and tylosine, but doxycycline had a better effect on LI excretion after treatment, total bacterial load and diarrhoea prevalence. A previous study showed that treatment with 8 mg tylosine tartrate per day for 7 days via drinking water could reduce the clinical signs and lesions and improve the rate of growth in nursery pigs challenged with LI (Paradis et al., 2004). In a field study from 2000 in Greece, in-feed treatment of 250 ppm doxycycline for 14 days significantly reduced the prevalence of LI and diarrhoea and improved the rate of growth, thus supporting the results of the current study (Kyriakis et al., 2002). Although this study demonstrated no difference in ADG between doxycycline or tylosine treatments, the results showed that high-level LI remains in faeces after treatment with tylosine. Tylosine tartrate and chlortetracycline have shown high intracellular and extracellular activity against LI (Wattanaphansak et al., 2009; Yeh et al., 2011). The high level of LI found in this study 2 days after treatment with tylosine was therefore surprising. The authors are not aware of any research into the antimicrobial susceptibility of doxycycline against LI to support the findings of doxycycline effectiveness in reducing LI excretion presented in this study. However, the effect of oxytetracycline treatment has recently been demonstrated. In a randomised clinical trial, 5 days of water medication with a dose of 5 mg to 20 mg oxytetracycline per kg bodyweight resulted in reduced diarrhoea and LI excretion after treatment (Larsen et al., 2016).

The application of medication via a water trough could influence the results since the pigs also had access to fresh water during the medication period, and might have preferred the fresh water to the medicated water. However, both antibiotic compounds were administered in the same way, making a comparison reasonable. A limitation of the study is the short study period of 14–35 days

after weaning. All pigs received only 5 days of antibiotic treatment throughout this period and therefore the lowest amount of antibiotics were used in pigs with a lower bodyweight that were treated early. Due to the risk of antibiotic treatments against enteric diseases recurring after the study period, it was not possible to evaluate the effect of the four treatment strategies on the rate of growth and total antibiotic usage for the whole nursery period of approximately 8 weeks after weaning. The aim of this study was to evaluate the effects of antibiotic treatment on reducing intestinal infections and thereby improving productivity. Other factors that can reduce the infection pressure, such as improvement of management and biosecurity, were not investigated.

Difference in concentration of the two types of drugs was the main reason why the study was not blinded. The parameter "weight before treatment" was used to calculate the correct dose of either doxycycline or tylosine and thereby made blinding difficult. To eliminate investigator bias we used objective parameters for our outcome variables; body weight and faecal dry matter measured using a scale, and bacterial intestinal pathogens demonstrated by qPCR.

This study demonstrated that diagnosing intestinal infections in groups of pigs before clinical signs are evident using pooled pen floor samples tested by qPCR can be used to support the decision for initiation of antibiotic treatment. Diagnosing intestinal infections by this method gives the advantage of achieving a better productivity and avoiding unnecessary treatments, thereby reducing the antibiotic usage to minimize the development of antibiotic resistance.

Three commercial pig herds were used in this study which was representative for typical Danish productions of nursery pigs in accordance to factors which could influence the conclusion of the study; health status, enteric pathogen profile, usage of in-feed Zink oxide, feeding strategy, and antibiotic usage. Variation within herds and within the EU swine population of the listed factors should be taken into consideration when interpreting the conclusions of this study.

5. Conclusion

The strategy resulting in the highest ADG was treatment 14 days after weaning in pens where *Escherichia coli* F4, F18, *Lawsonia intracellularis* or *Brachyspira pilosicoli* were detected by qPCR. Median diarrhoea pen-level prevalence at this time point was 0.09. There was no significant difference in ADG between treatment with doxycycline or tylosine, yet doxycycline was more effective in reducing LI excretion and diarrhoea prevalence after treatment.

Competing interests

None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of this paper.

Authors' contributions

JPN, CFH, KP and NW designed the sampling protocol and selected methods; CH conducted the qPCR analyses; NW performed the data sampling; MD and NW performed the statistical analysis; JPN, CFH, KP, MD, CH and NW devised the study and drafted the manuscript. All authors contributed to finalising the manuscript. All authors read and approved the final manuscript.

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